

REMARKS

Claims 1 to 20 were pending in this application prior to entry of the above amendments. Claims 1, 6, 8, 12, and 17 were amended; claims 3 to 5, 14 to 16 and 18 to 20 were cancelled; and new claims 21 to 30 were added. Because the number and dependency of the cancelled claims equals those added, no fees are due or presented herewith. However, if the Commissioner deems any fees due, he is hereby authorized to charge Yale Deposit Account Number 25-0110 for any fee deficiencies. Allowance of the amended claims is requested in view of these remarks, which address issues raised by the Examiner, Dr. Kerr, in the order she presented them in the above-mentioned Action, with cross-referencing to her numbered sections.

This invention relates to the treatment of a variety of central nervous system injuries by administration to a patient in need of axon regeneration a composition containing an effective amount of at least one rho protein inhibitor in amounts effective to inhibit rho or rac and stimulate neurite outgrowth.

2. Election. Whereas applicant does not agree with the Examiner's assessment that his originally presented claim set contained three distinct inventive concepts, the withdrawn claims have been cancelled to avoid argument on this point and advance prosecution of the case.

5. Drawings. In reply to the Notice of Draftperson's Patent Drawing Review (Form PTO 948) accompanying the Action, Replacement Figures 1 to 8 are enclosed with this response.

6. Title. The title was objected to as not being adequately descriptive of the instant subject matter. The words "Using Rho Protein Inhibitors" were added at the Examiner's suggestion.

7. Abstract. The specification was objected to for not containing an abstract on a separate sheet. A copy is enclosed herewith, which is a word processed version of the this application's abstract published by WIPO on 25 February 1999. The PCT cover sheet evidencing the identity of the paragraphs is attached hereto as Exhibit A.

8. Rho Protein Description. The description in Example 1 of wild type rho protein and the rho protein derivatives set out in the specification on page 13 at lines 20 to 28 was objected to as lacking specificity. The objection is respectfully traversed. The proteins employed by applicant are fully disclosed by the Nobes and Hall reference cited two lines above (*i.e.*, line 18), which is listed in the specification in the bibliography on page 26 (Nobes, C.D., and Hall, A., 1995, *Cell* 81: 53-62, attached hereto as Exhibit "B") and incorporated by reference on page 27. The proteins were prepared as set out in the paper using standard biochemical techniques, and provided to applicant by Dr. Hall for the experiments set out in Example 1.

9. Abbreviations. The specification was objected to for using 4 abbreviations not defined. Definitions were added to the specification in response to the objection.

10. Non-elected Subject Matter. Claims 1, 2, and 6 to 11 were objected to for containing non-elected subject matter. In response to this rejection, parent claim 1 was amended to particularly point out administration of a protein composition as suggested by the Examiner.

11. Improper Dependency. Claim 6 was objected to as being of improper dependent form for failing to further limit the subject matter of a previous claim. The claim was amended for reasons set out below, and the amendment renders the objection moot.

12. Claim Rejections Under 35 U.S.C. § 112. Claims 1, 2, 6, and 9 to 11 were rejected under the statute for failing to particularly point out what is meant by a "rho

protein inhibitor". Parent claim 1 was amended to clarify that applicant's treatment method for axon regeneration is directed to the administration of compositions containing an effective amount of a rho protein inhibitor in amounts effective to inhibit rho or rac and stimulate neurite outgrowth. Support for the amendment can be found in the specification on page 10 at lines 14 to 15, page 13 at line 9, page 16 at lines 4 to 22, page 19 at line 10 to page 22 at line 10, page 22 at lines 13 to 17 and in the legends of Figures 2, 6, and 11.

13. Claims 8 and 17 were rejected under the statute as being indefinite about in that the Examiner finds "C2/C3 inhibitor" unclear. The claims were amended in response to the rejection to particularly point out that the construct described by Barth, *et al.*, *Infect. Immun.* 66: 1364-1369 (1998) has the actin ADP-ribosylation activity deleted from the C2 toxin and the C3 enzyme activity substituted therefor. Support for the amendments can be found in the specification on page 11 at lines 15 to 17.

14 & 15. Claims 1, 2, and 6 to 11 were rejected under the statute as not enabled. The rejection is respectfully traversed, but the claims were amended to particularly point out that methods of the invention involve administration of at least one rho protein inhibitor in amounts effective to inhibit rho or rac and stimulate neurite outgrowth. Support for the amendments can be found in the specification on page 10 at lines 6 to 17. New claims 21 to 30 were added to particularly point out different embodiments of this illustrated in Examples 1 and 2.

It is Applicant's opinion that rho protein inhibitors are directed to a narrow group of proteins. Human genome data now available shows the entire genome to have about 30,000 to 100,000 genes. Of this number only about 50 are GTP-binding proteins. There are 2 classes of these: heterotrimeric proteins having 3 subunits and monomeric proteins having 1 subunit. There are only 5 or 6 of this latter family, and the rho family is only one subfamily within that group. Please note the findings reported in Nobes and Hall, cited above and in the specification, and attached hereto as Exhibit "B"). So

targeting rho is quite selective. And C3 is specific and reacts with no other protein in the body.

Applicant also takes issue with what seems to be the Examiner's assertion that DRG neurons are just peripheral. DRG neurons send 1 axon into the periphery and one to the brain. So DRG neurons ~~or central or peripheral~~^{are}. Applicant has observed this, as have Västrik, *et al.*, cited in the Information Disclosure Statement (*Current Biology* 9:991-998, 1999). That reference and Lehmann, *et al.*, paper also cited in the IDS (*J. Neurosci.* 19:7537-7547, 1999) confirm the scope of Applicant's invention and show the *in vivo* efficacy of C3 exoenzyme to promote CNS axon regeneration. In addition, the Lehmann paper sets out an assay that can be used for identifying useful inhibitors of the invention. Data in Pasterkamp, *et al.*, *Molecular and Cellular Neuroscience* 13: 143-166, 1999, attached hereto as Exhibit "C" also further support the claimed invention, and confirm Applicant's findings. Given this experimental support for the invention and the current interest in the field, Applicant believes the claims are amply enabled for skilled workers, and the practice of the claimed invention is straightforward.

16. Claim Rejections Under 35 U.S.C. § 102(b). Claims 12 and 13 were rejected as anticipated by Morii, *et al.* (*J. Biochem.* 107: 769-775, 1990). The claims were amended, converting them from composition claims to method claims in response to the rejections. As amended, the claims should be allowable because Morii, *et al.* do not anticipate Applicant's method of stimulating axon regeneration using rho protein inhibitors.

17. Claim Rejection Under 35 U.S.C. § 103. Claim 17 was rejected as unpatentable over Barth, *et al.*, (*Infect. and Immun.* 66: 1364-1369, 1998) in view of Morii, *et al.* The claim was amended to become a method claim. It should be allowable for reasons discussed in the previous paragraph, namely that the references do not anticipate or suggest Applicant's claimed method of regenerating axons.

Applicant has made a significant advance for the treatment of acute or chronic spinal cord injury, white matter stroke, and traumatic brain injury. Reconsideration and allowance of the amended claims is therefore respectfully requested. If the undersigned can advance the prosecution of this application in any way whatsoever, please call at the number listed below.

Respectfully submitted,

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Marked Up Version of Amendments Required by 37 C.F.R. § 1.121

Specification Paragraph on Page 3, Lines 16 to 28:

The semaphorin/collapsin family of proteins has been recognized as one important negative regulator of axon outgrowth and terminal arborization (Luo, *et al.*, 1993; Kolodkin, *et al.*, 1992, 1993). Chick collapsin-1 induces growth cone collapse and a cessation of neurite outgrowth from at least a subset of dorsal root ganglion neurons (Raper and Kapfhammer, 1990; Luo, *et al.*, 1993; hereinafter abbreviated "DRG"). Insect semaphorins have a demonstrated *in vivo* role during axonal pathfinding and synaptic terminal branching (Kolodkin, *et al.*, 1992; Matthes, *et al.*, 1995). There are at least 7 vertebrate semaphorins identified and there may be as many as 20 members of this family (Puschel, *et al.*, 1995; Messersmith, *et al.*, 1995; Luo, *et al.*, 1995; Inagaki, *et al.*, 1995; Adams, *et al.*, 1996). A decrease in actin filaments after collapsin-1 application has been documented (Fan, *et al.*, 1993). The mechanisms whereby collapsin-1 binding to an unidentified transmembrane receptor triggers this depolymerization is unclear.

Specification Paragraph on Page 4 at Lines 1 to 9:

In non-neuronal cells, the rho subfamily of monomeric ras-related GTP-binding proteins have prominent effects on the actin-based cytoskeleton and on cell shape (Hall, 1990; 1994). In fibroblasts, rho activation has been linked to stress fiber formation and focal adhesions, rac1 activation with membrane ruffling and lamelipodia, and cdc42 activation with filopodial formation (Nobes and Hall, 1995). Single amino acid substitutions have been identified which produce constitutively active or dominant negative forms of each of these proteins. The C3 transferase from Clostridium botulinum (hereinafter abbreviated C. botulinum) ADP-ribosylates rho specifically and inactivates the G protein.

Specification Paragraph on Page 15 at Lines 5 to 16:

Comparison of collapsin-1 action with LPA and thrombin action. As a first step to assessing the role of small G proteins in collapsin action, the effect of readily available

pharmacological agents on collapsin-1 action was compared to their effects on LPA and thrombin action. The myosin light chain kinase inhibitor, KT5926, blocks LPA-induced neurite retraction and also decreases the potency of recombinant collapsin-1 as a growth cone collapse factor (Figure 1A). A number of other agents had little or no effect on collapsin-1 action including tyrosine kinase inhibitors, protein kinase A inhibitors, voltage-sensitive Ca channel blockers and depolarization with KCl. The more general protein kinase inhibitor, staurosporine, and the protein kinase C activator, tissue plasminogen activator (hereinafter abbreviated "TPA"), both induced growth cone collapse at concentrations below 10 nM, but their action was not synergistic with collapsin-1.

Specification Paragraph on Page 20 at Line 20 to Page 21, line 4:

Mechanism of rac1 activation: dbl proteins, G protein cascade, Collapsin Response Mediator Protein (CRMP). The mechanism by which rac1 might be activated by extracellular collapsin-1 is unclear. In other cell types, proteins with domains homologous to the human Dbl act upstream of rac1 as guanine nucleotide exchange factors (Boguski and McCormick, 1993), but their presence in neuronal growth cones has not been studied. Receptors of several classes appear to be capable of activating rac1 in other cells, including receptor tyrosine kinases, serpentine receptors coupled to heterotrimeric G proteins and cytokine receptors of the TNF class. A central role for heterotrimeric G proteins in growth cone signal transduction is supported by a number of studies (Strittmatter, *et al.*, 1990; 1993; 1994b; 1995). Data presented here indicate that heterotrimeric G proteins (Figure 1B) may be involved in collapsin signaling. An intracellular family of neuronal proteins, CRMPs, has been identified; these are required for collapsin action but their interaction with other members of this signaling pathway is not established (Goshima, *et al.*, 1995; Wang and Strittmatter, 1996). There are no data indicating that intracellular calcium ion levels are likely to mediate collapsin action.

Claim 1 (Amended). A method for promoting central nervous system axon growth in a patient in need of axon regeneration comprising administering to the patient a composition

containing an effective amount of at least one rho protein inhibitor in amounts effective to inhibit rho or rac and stimulate neurite outgrowth.

Claim 6 (Twice Amended). A method according to claim 1 wherein the [rho protein inhibitors are selected from the group consisting of rho, rac, and cdc42 iinhbitors, and mixtures thereof] inhibitor inhibits a rac protein.

Claim 8 (Amended). A method according to claim 24 wherein the [patient is treated by admiinistration of] rho protein inhibitor is a chimeric *C. botulinum* C2/C3 [inhibitor to the patient] construct having the actin ADP-ribosylation activity deleted from the C2 toxin and the C3 enzyme activity substituted therefor.

Claim 12 (Amended). A method for promoting central nervous axon growth in a patient in need of axon regeneration by administering to the patient a pharmaceutical composition [for treatment of central nervous system injury comprising] containing at least one rho protein inhibitor selected from the group consisting of a rac protein, a rho protein, a protein that inhibits both a rac protein and a rho protein, and mixtures thereof, in amounts effective to inhibit rho or rac such that neurite outgrowth is stimulated [in a pharmaceutically acceptable carrier].

Claim 17 (Amended). A method [composition] according to claim 12 [which] wherein the composition comprises a chimeric C2/C3 *C. botulimun* exoenzyme contruct having the actin ADP-ribosylation activity deleted from the C2 toxin and the C3 enzyme activity substituted therefor.